Lathyritic Changes in Carp Caused by β-Aminopropionitrile and D-Penicillamine

Mamoru SATO*, Reiji YOSHINAKA*, and Shizunori IKEDA*

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Carp were fed diets containing either 0.3% β-aminopropionitrile (β-APN) or 0.3% D-penicillamine. On the 67th day of the feeding, skeletal deformity was identified in 25% of the fish given β-APN and 32% of those given D-penicillamine. X-ray examination of the abnormal fish showed severe spinal curvature at the region of 12th to 16th vertebrae, separation and fracture of the vertebral column, deformation of the ribs, and fracture of the rib in its basal part. In addition, fish given these drugs exhibited hemorrhage in the fins, the skin, and the viscera, and fragility of the connective tissues.

Carp, when injected intraperitoneally with these drugs, also showed similar toxic symptoms.

These experimental results indicated that β-APN and D-penicillamine caused lathyritic changes in carp similar to those seen in rats and chickens.

Recently as the cultivation of various species of fish has grown remarkably in our country, skeletal deformity often seen in cultured fishes has become a problem awaiting solution. Experimental skeletal abnormalities observed in fish fed certain types of experimental diet or administered certain kinds of drug have been reported by many investigators. KITAMURA et al.1) demonstrated that rainbow trout, Salmo gairdnerii, showed spinal curvatures when fed vitamin C-deficient diet. HALVER et al.2) observed acute lordosis and scoliosis in coho salmon, Oncorhynchus kisutch, and rainbow trout on vitamin C-deficient diet. LOVELL3) made similar observations with channel catfish, Ictalurus punctatus, fed the diet without supplemental vitamin C. MURAKAMI4) demonstrated that lack of mineral in diets caused skeletal deformity in carp, Cyprinus carpio. KUBOTA et al.5) and MATSUISHIMA et al.6) showed that the administration of sulfa drugs caused fracture of the vertebral column in yellow-tail, Seriola quinqueradiata. KITAMURA7) showed that semicarbazide induced skeletal deformity in rainbow trout, red salmon, Oncorhynchus nerka, dog salmon, Oncorhynchus keta, and carp when administered orally. KANAZAWA8) reviewed deformity of the vertebral column of fishes induced by agricultural chemicals. However, the underlying mechanisms of these deformation still remain unsolved from the biochemical point of view.

Lathyrisn designates a diseased state in animals induced by feeding the seed of sweet pea, Lathyrus odoratus. Experimental lathyrisn can be caused by a number of synthetic compounds, such as aminoacetonitrile, β-aminopropionitrile (β-APN), and semicarbazide, which are called lathyrogens.9,10) Lathyrogens produce marked skeletal changes,11-14)
consisting of malformation of the long bone, spinal curvature, and deformation of the epiphysial discs, and other connective tissue abnormalities. These effects of lathyrogens on the skeletal system are specifically referred to by the term osteolathyrism.

It has been demonstrated that penicillamine at toxic levels also has osteolathyrigenic activities similar to those seen in β–APN.

Even though experimental lathyrism in fish may be not concerned directly with skeletal deformity of fishes which has been observed by many investigators, to study on experimental lathyrism in fish is important so as to give a clue to the elucidation of causes of skeletal deformity. The present paper describes a diseased state in connective tissues of carp given β–APN or D-penicillamine.

Materials and Methods

Reagents β–APN was obtained from Tokyo Chemical Industry Co. Ltd., and D-penicillamine from Nutritional Biochemicals Company, U. S. A. Other reagents were of analytical grade.

Oral administration of β–APN and D-penicillamine to carp Young carp were accustomed to control diet (see in Table 1) for 4 weeks. They (35 g of average body weight) were then divided into three groups, with fifty carp in each group, and kept in aquariums (500 l) supplied with dechlorinated city water at 22–25°C. The fish were given the test diets as moist pellets for 67 days. They were fed twice daily at the level of 3% (as dry base) of body weight per day. The composition of the test diets is shown in Table 1.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>β–APN</th>
<th>D-Penicillamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>45g</td>
<td>45g</td>
<td>45g</td>
</tr>
<tr>
<td>Potato starch*¹</td>
<td>48</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mineral mixture*²</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin mixture*³</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>β–APN</td>
<td>—</td>
<td>0.3</td>
<td>—</td>
</tr>
<tr>
<td>D-Penicillamine</td>
<td>—</td>
<td>—</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*¹ Potato starch was mixed with other ingredients after heating with water in a boiling water bath.

*² McCollum’s salt mixture No. 185.

*³ The formula of vitamin mixture by HALVER et al.

Intraperitoneal administration of β–APN and D-penicillamine to carp Each of ten carp (65 g of average body weight) which had been given the control diet for 7 weeks was injected intraperitoneally with 0.1 mmole of either β–APN or D-penicillamine/100 g of body weight daily for 3 days. After the administration of the drugs was discontinued for next 4 days, they were again injected with each drug in same manner for 3 days. Feddng con-
ditions were the same as the above-mentioned and the control diet was given to each group.

**X-ray examination of the skeletal system** In order to assess the skeletal deformity and to examine the mode of damage, X-ray surveys of the skeletal system were made on all of the fish by using X-ray apparatus (Koizumi X-Senkosha Co. Ltd., Tokyo).

**Results**

**Oral administration of β-APN and D-penicillamine** Growth curves in per cent weight gain of fish fed test diets are shown in Fig. 1. After about 10 days, fish given β-APN began to exhibit loss of appetite, followed by retardation of growth compared with that of the control group. Fish given D-penicillamine had a good appetite and gained more weight than the control group throughout the experimental period.

![Fig. 1. Growth curves of carp fed the test diets. Carp, weighing 35 g on the average at the beginning of the experiment, were used.](image1)

![Fig. 2. The development of skeletal deformity in carp given orally β-APN or D-penicillamine.](image2)

On the 9th day of the experiment, the manifestation of skeletal deformity was noted in some fish of both drug-treated groups. After that, the number of the deformed fish induced by β-APN or D-penicillamine gradually increased as shown in Fig. 2. At the end of the experimental period, skeletal deformity was identified in 25% of fish given β-APN and 32% of fish given D-penicillamine. No mortality was observed in any of the groups until the end of the experiment.

Fig. 3 shows radiographs of some deformed fish induced by β-APN or D-penicillamine. As seen in the radiographs, the deformed fish in both treated groups showed severe deformity of the vertebral column, especially marked changes at the region of 12th to 16th verte-
brae. In addition to these changes, separation and fracture of the vertebral column, deformation of the rib, and fracture of the rib in its basal part were observed in some abnormal fish given β-APN or D-penicillamine.

Other symptoms observed in fish given these drugs are summarized in Table 2. Fish given drugs exhibited hemorrhage in the caudal and dorsal fins and in the kidney; fragility of the connective tissues, especially a decrease in the mechanical strength of the caudal fin, the peritoneum, and the gall bladder. These indicate that β-APN and D-penicillamine act mainly upon the connective tissues.

In addition, fish given D-penicillamine showed nervous disorders, and some of the fish exhibited ataxia and epileptiform fit on the 10th day. These behaviors resemble those of vitamin B₆ deficient carp observed by OGINO, KUCHINSKAS and DU VIGNEAUD, and ASATOMOR demonstrated that L- and D-penicillamine cause a depletion of vitamin B₆ when administered to rats. It seemed appropriate to examine whether vitamin B₆ deficiency-like symptoms in penicillamine-treated carp can be relieved by the administration of pyridoxal phosphate. Some of the fish which exhibited epileptiform fit were given orally 6 mg of pyridoxal phosphate/100 g of body weight daily for 2 days. By this treatment, they soon recovered from epileptiform fit. This indicates that nervous disorders in carp given D-penicillamine might be induced by a depletion of vitamin B₆ which was caused by the administration of the drug. Therefore, fish receiving D-penicillamine were given the diet supplemented with 10 mg of pyridoxal phosphate/100 g of diet on and after the 13th
day of the experimental period. With this treatment, symptoms of vitamin B<sub>6</sub> deficiency were not observed in this group.

**Intraperitoneal administration of β-APN and D-penicillamine** Toxic symptoms observed in fish injected intraperitoneally with β-APN or D-penicillamine are summarized in Table 3. Within 20 to 30 min after the injection of β-APN, dark coloration, or an ecchymosis, in the skin from posterior part of the dorsal fin down to the anal fins was observed. At about 24 hr after the injection, the fish showed an erosion of the skin around injected part. On the 10th day hemorrhagic erosion and ulceration of the muscular layer

<table>
<thead>
<tr>
<th>Experimental day</th>
<th>β-APN group</th>
<th>D-Penicillamine group</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Hemorrhage in the caudal fin and the kidney; poor appetite.</td>
<td>Ataxia; epileptiform fit; hemorrhage in the caudal fin; fragility of the peritoneum and the gall bladder.</td>
</tr>
<tr>
<td>32</td>
<td>Hemorrhage in the caudal and dorsal fins, the spleen, and the kidney; fragility of the caudal fin; dark green coloration on the gall bladder; poor appetite.</td>
<td>Hemorrhage in the caudal and dorsal fins, the spleen, and the kidney; abnormal swimming in some fish.</td>
</tr>
<tr>
<td>67</td>
<td>Hemorrhage in the fins; fragility of the caudal fin, and the gall bladder; green coloration on the hepatopancreas; poor appetite.</td>
<td>Fragility of the caudal fin, the gall bladder, the swim bladder, and the peritoneum; yellowish brown coloration on the hepatopancreas.</td>
</tr>
</tbody>
</table>

**Table 2.** The toxic symptoms in carp given orally β-APN or D-penicillamine

<table>
<thead>
<tr>
<th>Experimental day</th>
<th>Drug administration</th>
<th>β-APN group</th>
<th>D-Penicillamine group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>Dark coloration in anterior part of the tail skin.</td>
<td>Patched discoloration of the head and the fins.</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>Erosion of the skin around injected part.</td>
<td>Hemorrhage in the fins.</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>Erosion of the skin in the eyes and the fins; death of two fish.</td>
<td>Hemorrhage in the basal part of the fins.</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>Erosion and ulceration of the skin in abdominal region; death of one fish.</td>
<td>Erosion in the basal part of the caudal fin; death of two fish.</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>Internal hemorrhage in the head and the trunk skin.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>Fragility of the caudal fin.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>Erosion and ulceration of the skin in abdominal region; death of one fish.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>Death of one fish.</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td></td>
<td>Exfoliation of the scale.</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>Erosion and ulceration of muscular layer in abdominal region; one deformed fish.</td>
<td></td>
</tr>
</tbody>
</table>
under the skin were observed in the abdominal region of β-APN-treated fish. In this group, four fish died and one fish showed skeletal deformity until the end of the experimental period.

Within 30 min after the first injection of D-penicillamine, the fish showed either a patched discoloration or an internal hemorrhage in the head and the fins. The hemorrhage disappeared after 5 hr. At about 24 hr after the injection, hemorrhage was again observed in the pectoral, dorsal, and caudal fins of all the treated fish. On the 4th day of the experiment, the fish exhibited patched discoloration in the head and the trunk skin, the hemorrhagic fins, and erosion in the basal part of the fins. In this group, two fish died and no fish showed skeletal deformity during the 10 days of the experimental period.

**Discussions**

Experimental skeletal abnormalities in various species of fish have been observed by many investigators,1-8) but their underlying mechanisms remain almost unsolved.

It has been demonstrated that marked skeletal deformity develops in rats and chickens given β-APN,11-15) a typical lathyrogen, and penicillamine,16,17) a lathyrogenic compound. These changes were shown to be quite specifically related to a disturbance of collagen metabolism.15,16,22,23)

Even though there is no overall similarity between the pathology of lathyritic animals and the pathology of deformed fishes observed by many workers, it seems reasonable to assume that an abnormality of collagen metabolism may be concerned in the manifestation of the deformity in fishes as one of the causes. However, little attention has been paid to effects of lathyrogens and penicillamine on fishes. Kitamura7) demonstrated that semicarbazide, one of lathyrogens, induced osteolathyrism in rainbow trout, red salmon, dog salmon, and carp when administered orally to them, but its underlying mechanism still remains unexplained.

In the present study it was demonstrated that β-APN and D-penicillamine induce typical lathyrism or various toxic symptoms in carp, such as marked skeletal deformity, hemorrhage in various tissues, and fragility of the fins and the peritoneum. Skeletal changes might be caused by a reduction in the mechanical strength of the bone accompanying a collagen defect induced by β-APN and D-penicillamine; other toxic symptoms might also reflect an abnormal pattern of collagen metabolism. To make sure of this view, further studies were undertaken. The details will be reported in a subsequent paper.

Seifert et al.24) reported that the feeding of large doses of penicillin G to rats caused the collagen maturation defect which was reflected by decreased breaking strength of unwounded and wounded skin. This change was thought to be mediated through one or more naturally occurring metabolites of penicillin G, such as penicillamine. They advised that their data should not be construed as indicating that penicillin as used for the treatment
of patient interferes with collagen metabolism, since extremely high doses of penicillin G were used in their experiments. Nevertheless, we must call our attention to their results in connection with the fact that many kind of drugs are used for the prevention and treatment of various diseases in cultured fishes.

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References